

# **Human Innate Lymphoid Cells (ILCs): towards a uniform immune-phenotyping**

**Running Title: hILC phenotype**

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## Abstract

Helper Innate Lymphoid Cells (ILCs), the most recently identified population of the Innate Lymphoid Cell family, play a fundamental role in the restoration of tissue integrity, in the protection against infiltrating pathogens as well as in tumor immune-surveillance. ILCs have been divided into three main subsets, ILC1, ILC2 and ILC3, that can be specifically activated by different signals coming either indirectly from pathogens or from other cell populations, including cancer cells. Following activation, ILCs are in turn able to promptly secrete a wide range of soluble mediators that modulate effector cell functions. The discovery and the study of these immune cells is now offering important opportunities for innovative therapies of allergic airway diseases, inflammatory disorders and might be crucial for the discovery of new targets for the therapy of cancer. It is therefore fundamental that the scientific community establishes harmonized guidelines to obtain a consensus in the identification and phenotypical and functional characterization of ILCs.

## Introduction

Beside natural killer (NK) cells and lymphoid tissue inducer (LTi) cells, discovered respectively in 1975 and 1997(1,2), several distinct Innate Lymphoid Cell (ILCs) populations have been recently identified(3). As NK and LTi, ILCs require the common  $\gamma$  chain of the interleukin-2 (IL-2) receptor and the transcriptional repressor Id2 for their development, and are characterized by the absence of rearranged antigen-specific receptors(4-6). These ILC populations have been named ILC1, ILC2 and ILC3 according to their cytokine and transcriptional profiles that mirror the ones of CD4<sup>+</sup> T helper (Th)1, Th2 and Th17/22 cells, respectively, and are therefore considered “helper” ILCs(7,8). According to this classification, LTi have been included in the ILC3 population, while NK cells are part of the ILC1 subset, even if they represent the “cytotoxic” ILCs, being the counterpart of CD8<sup>+</sup> cytotoxic T cells(9) (**Fig.1**).

Helper ILCs are circulating in the peripheral blood but are also present in lymphoid organs and are enriched at mucosal and barrier surfaces. As innate immune cells, their role is crucial during the early stages of the immune responses(10-12), when they constitute the main source of several pro- and anti-inflammatory cytokines (**Fig.1**). Since ILCs are now the focus of extensive investigations describing their central role in both homeostatic and pathophysiological processes, a careful identification of their phenotype as well as of their function will be key for their understanding.

## Identification of ILCs

The first concern in the study of ILCs is represented by the fact that several groups have used distinct criteria and markers for their identification. Indeed, ILCs have been initially defined as Lineage negative (Lin<sup>-</sup>) CD127<sup>+</sup> cells, but some groups have recently identified non-classical human ILCs CD127<sup>dim</sup> and mouse ILCs CD127<sup>-</sup> (that are out of the focus of this review)(13-16).

Moreover, it is difficult to determine what “Lineage” means for the different research teams. In humans, the most common Lineage markers include CD3, CD19, CD56, CD14, HLA-DR and CD34, to respectively identify T, B and NK cells, monocytes, dendritic cells and hematopoietic progenitor cells. However, these markers might not be sufficient to exclude Lineage positive cells from the ILC gate. For example, it is known that CD3 can be internalized and, therefore, its extracellular expression consequently be reduced once T cells get activated(17), opening the possibility that CD127<sup>+</sup> T cells could be included in the CD3<sup>-</sup> gate and, thus, making CD3 insufficient for T cell exclusion. Moreover, CD19 might also not be sufficient to completely exclude all human B cell subsets(18), while CD56 and HLA-DR should not be included in the Lineage being expressed on a subset of ILC3(19).

Since there is not a way to precisely determine which markers belong to the “Lineage”, different groups have developed their own “Lineage mix”. As a consequence, results obtained from different groups are not comparable and/or reproducible. To test the impact of using different Lineage mix on the reproducibility of the results, we collected blood from three healthy donors and we compared the frequency of total ILCs obtained with our Lineage mix and with the mix published by four other groups. As shown in **Figure 2**, by using different Lineage mix, the frequencies of gated ILCs are indeed different, irrespective of inclusion or exclusion of the CD127<sup>dim</sup> ILCs.

### **The choice of the Lineage mix**

To determine which markers need to be included in an optimal Lineage mix we started with a basic cocktail comprising CD3, CD4, CD8, CD14, CD15, CD16, CD19, CD20 and CD34 and we tested which cells were included in the Lin<sup>-</sup>CD127<sup>+</sup> region, that were not *bona fide* ILCs.

Since dendritic cells are identified as Lin<sup>-</sup>HLA-DR<sup>+</sup> cells, but HLA-DR can also be expressed by ILCs(19), we assessed the expression of other DC markers in the Lin<sup>-</sup>CD127<sup>+</sup> population. The two main populations of myeloid DCs are CD11c<sup>+</sup>CD16<sup>+</sup> or CD11c<sup>+</sup>CD1c<sup>+</sup>, while plasmacytoid DCs are CD11c<sup>-</sup>CD123<sup>+</sup>(20). Since CD16 was included in our mini-cocktail, we tested only CD1c and CD123. As shown in **Figure 3A**, by comparing Lin<sup>-</sup>CD127<sup>-</sup> and Lin<sup>-</sup>CD127<sup>+</sup> cells, we did not observe a population of CD1c<sup>+</sup> or CD123<sup>+</sup> in the ILC gate. Notably, for dendritic cell exclusion, beside CD1c<sup>+</sup> or CD123<sup>+</sup>, we decided not to include CD11c in the Lineage since ILCs are developmentally related to NK cells and it has been shown that NK cells can express this marker(21).

Secondly, we wanted to address if basophils or mast cells were contaminating the ILC gate. To test this hypothesis, we monitored the expression of Fc RI and CD203c in the Lin<sup>-</sup>CD127<sup>-</sup> and in the Lin<sup>-</sup>CD127<sup>+</sup> gates(22). As shown in **Figure 3A**, we found both a Fc RI<sup>+</sup> and a CD203c<sup>+</sup> population in the ILC gate, and therefore we considered necessary to include these two markers in our Lineage mix.

Finally, we tested if myeloid derived suppressor cells (MDSCs) were present in the gate of ILCs. Since we had already included CD14 and CD15 in the Lineage cocktail, we only verified the expression of CD33 in the Lin<sup>-</sup>CD127<sup>+</sup> and in the Lin<sup>-</sup>CD127<sup>-</sup> regions(23). As shown in **Figure 3A**, we found a CD33<sup>+</sup> population in the ILC gate and we thus included CD33 in our Lineage cocktail. Using this Lineage mix, the presence of ILCs within the Lin<sup>-</sup>CD127<sup>-</sup> population is negligible, as assessed by evaluating the expression of T-bet, GATA3 and RORγt in Lin<sup>-</sup>CD127<sup>-</sup> cells (**Figure 3B**).

In conclusion, by performing all these necessary, but possibly not exhaustive comparisons, we determined that a Lineage mix useful to exclude rare populations from the ILC gate should

contain at least antibodies against CD3, CD4, CD8, CD14, CD15, CD16, CD19, CD20, CD33, CD34, CD203c and Fc RI.

#### **CD161 and NKp44: should we use them?**

Another concern in the identification of human ILCs is represented by the use of the marker CD161. Indeed, some groups showed that all ILCs are CD161<sup>+</sup> and use this marker in their gating strategy(24,25), while others, including our group, do not add CD161 in the gating strategy(26-28). Since it was shown that CD161 is not present in all ILC subsets, being at least ILC1 and LT $\alpha$  CD161<sup>+/-</sup>(7,29), we propose not to include this marker in the gating strategy, thus avoiding to exclude from the analysis ILCs that are CD161<sup>-</sup> (**Fig.4**).

Concerning the phenotype of the three different human ILC subsets, namely ILC1, ILC2 and ILC3, it is well established that ILC1 are CRTH2<sup>-</sup>ckit<sup>-</sup>CD56<sup>-</sup>, ILC2 are CRTH2<sup>+</sup>ckit<sup>+/-</sup>CD56<sup>-</sup>, while ILC3 are CRTH2<sup>-</sup>ckit<sup>+</sup>CD56<sup>+/-</sup>(7,19). Here, the main concern is represented by the choice of the natural cytotoxicity receptor (NCR) used to discriminate between the two main subsets of ILC3, i.e. ILC3 NCR<sup>+</sup> and ILC3 NCR<sup>-</sup>. In humans, the NCR family is made of three molecules: NKp30, NKp44 and NKp46. However, only NKp46 is present in both humans and mice and is expressed regardless of the activation status of the cells(30). Therefore, even if several groups proposed to use NKp44 to define ILC3 NCR<sup>+</sup> cells(26,31), we and others suggested to use NKp46(27,28,30). Once again, the frequencies of ILC3 NCR<sup>+</sup> and ILC3 NCR<sup>-</sup> change according to the choice of the NCR marker used for their identification (**Fig.4**).

#### **The ILC gating strategy and the cytokine production**

The main function of ILCs is to rapidly respond to different signals by secreting a wide range of soluble mediators. In particular, ILC1 are activated by IL-12/IL-15/IL-18 to secrete type 1

cytokines (i.e. IFN- $\gamma$ , TNF- $\alpha$ ), ILC2 by IL-25/IL-33/TSLP/PGD2 to secrete type 2 cytokines (i.e. IL-4, IL-5, IL-13) and ILC3 by IL-23/IL-1 $\beta$  to secrete type 3 cytokines (i.e. IL-17, IL-22, GM-CSF, LT- $\alpha$ )(32). As a consequence, ILC1 are involved in type 1 immunity to viruses and intracellular bacteria(16,33), ILC2 in type 2 immunity to helminths and allergens(34,35), while ILC3 in type 3 immunity to extracellular microbes(36,37). Therefore, it is fundamental that the three different ILC subsets identified by the selected gating strategy are sensitive to the subset-specific stimulation and are able to produce the pattern of soluble mediators specific of each ILC subset (**Fig.5**). It has to be noticed that ILCs can also be stimulated with phorbol 12-myristate 13-acetate (PMA) and ionomycin to induce cytokine production/secretion. However, this stimulation is not subset-specific and therefore the different ILC subsets might display production of soluble mediators that is not occurring under physiological conditions (e.g. ILC2 able to produce IFN- $\gamma$ ).

### **The ILC gating strategy in disease**

Finally, the selected gating strategy should allow to evaluate phenotypic and functional alterations in ILCs in different disease settings. In that regard, it has been shown that ILC2 play a crucial role in allergic airway disease both in mice and in humans(38,39). In this setting, among other stimuli, ILC2 are enhanced and activated by eicosanoids, suggesting that ILC2 function could be modulated by interacting with metabolites of the eicosanoid pathway(40-42). Given the fact that ILC2 are identified as CRTH2<sup>+</sup> ILCs, but that CRTH2 is also expressed by basophils and mast cells(22), to appreciate the increase of ILC2 in allergic patients, it is important that the Lineage cocktail includes markers allowing the exclusion of these cells by using other markers than CRTH2 (e.g. Fc  $\epsilon$ RI and CD203c) (**Fig.6**).



It has been shown that ILCs also play a central role in cancer immunity(30,43,44). For example, in the context of leukemia, ILC1 are increased, although hypo-functional, being less able to produce type 1 cytokines in comparison to their healthy counterpart. In particular, ILC1 were found increased in patients suffering from either acute myeloid leukemia or chronic lymphocytic leukemia(27,45,46). Again, the choice of the Lineage cocktail and of the markers useful to identify ILCs should enable to appreciate the ILC1 increase in leukemic patients, without risking a contamination of the ILC1 subset by leukemic cells or other cell subsets (**Fig.6**).

### **Concluding remarks**

In the last few years, great efforts have been done to characterize the newly discovered family of helper ILCs in tissue and in peripheral blood, in both mice and in humans. However, data generated by different groups are often not comparable due to the choice of the markers used to identify both total ILCs and the different ILC subsets. In this review, we describe our gating strategy to identify human circulating ILCs, providing evidence to support our choice of markers (**Table 1**). However, we believe that a consortium of experts in the field should meet to reach a consensus about which markers have to be included in the Lineage and which ones have to be used to discriminate the different ILC subsets, both for mouse and human ILCs. This would be extremely important not only for fundamental research on ILCs, but also for the rapid application of these findings to translational research. Indeed, the next challenge in the ILC research field is represented by the identification of strategies to target ILCs in human diseases. Therefore, the increasing importance of ILCs in innate responses really deserves that a uniform phenotype is established.

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**Conflict-of-interest disclosure**

The authors declare no competing financial interests.

## Figure legends

### **Figure 1. Innate lymphoid cells and their counterparts in the adaptive immune response.**

Both the innate and the adaptive lymphocytes arise from the Common Lymphoid Progenitor (CLP). Helper ILC1, ILC2 and ILC3 and cytotoxic NK cells originate, upon cytokine stimulation, from the Early Innate Lymphoid Cell Progenitor (EILP). Helper Th1, Th2 and Th17 and the cytotoxic CD8<sup>+</sup> T cells originate from the CD4<sup>+</sup>CD8<sup>+</sup> Double Positive Progenitor (DP), upon T-Cell Receptor (TCR) and cytokine activation. The signature cytokines for each subset are indicated.

### **Figure 2. ILC frequencies in peripheral blood and tissues. A)**

Peripheral blood mononuclear cells (PBMCs) were purified by Ficoll density gradient from 3 healthy donors. Five different Lineage mixes to identify ILCs by multicolour flow cytometry were compared: i) Lineage ILC Mix: CD3 (UCHT1), CD4 (13B8.2), CD14 (RMO52), CD16 (3G8), CD19 (HD237) from Beckman Coulter, CD8 (LT8), CD15 (MEM-158) from AbD Serotech, CD20 (2H7), CD33 (HIM3-4), CD34 (561), CD203c (E-NPP3) and FcεRIα (AER-37) from Biolegend; ii) Mix 1(47): CD3, CD14, CD19, CD34; iii) Mix 2(48): CD3, CD14, CD19, CD20, CD56, CD11c (3.9) and CD123 (6H6) from Biolegend; iiiii) Mix 3(49): CD1a (BD, HI149) , CD3, CD11c, CD14, CD16, CD19, CD34, TCRαβ (IP26A), TCRγδ (B1) and BDCA2 (V24-785) from BD, FcεR1, CD123 and iiiiii) Mix 4(31): CD1a, CD3, CD11c, CD14, CD16, CD19, CD34, TCRαβ, TCR γδ, BDCA2, FcεR1, CD94 (Biolegend, DX22), CD123.

ILCs were identified within the peripheral blood lymphocyte region on the basis of their forward (FSC) and side scatter (SSC) profiles (FSC low and SSC low) and by excluding from the analysis doublets (FSC H/FSC W dot plot, followed by SSC A/SSC W dot plot) and dead cells (positive for ViViD LIVE/DEAD fixable dead cell stain kit (LifeTechnologies)). Left part:

representative comparison of ILC gating on the same donor according to the different Lineage mixes used. Total ILCs were gated in red as Lin<sup>-</sup>CD127<sup>+</sup> cells, while Lin<sup>-</sup>CD127<sup>bright</sup> ILCs were gated in blue. Right part: histograms represent the mean  $\pm$ SD of ILC frequencies using the different Lineage mixes. ILC frequencies can significantly differ depending on the Lineage mix used. **B)** Following CD45-Krome Orange (J.33, from Beckman Coulter) gating, the Lineage ILC mix was also used to identify total ILCs and ILC subsets in tonsils, spleen, axillary, mesenteric and inguinal lymph-nodes, thymus, liver, small intestine and colon from tissue cell suspension from freshly deceased, healthy cadaveric donors. Representative plots are shown. The different ILC subsets were determined according to the expression of CRTH2 vs cKit: ILC1 CRTH2<sup>-</sup>cKit<sup>-</sup>, ILC2 CRTH2<sup>+</sup>cKit<sup>+/+</sup> and ILC3 CRTH2<sup>-</sup>cKit<sup>+</sup>. Antibodies used: Lineage ILC Mix-FITC, CD127-PerCP-Cy5.5 or BV421(A019D5), cKit-PE or APC (104D2) from Biolegend, CRTH2-BV421 or PE-CF594 (BD, BM16).

The dot plots shown in this review are the result of a minimum of 10<sup>6</sup> MNCs acquired on a Gallios flow cytometer (Beckman Coulter). Data were analyzed using FlowJo<sup>TM</sup> software (TreeStar).

**Figure 3. The choice of the Lineage mix.** **A)** A minimal Lineage mix (Lin) was used comprising PE-conjugated CD3 (UCHT1), CD4 (13B8.2), CD8 (SFCI21Thy2D3), CD19 (89B) from Beckman Coulter, CD14 (M P9), CD15 (HI98), CD34 (8G12) from BD and CD16 (3G8) from Biolegend. CD127 was used in BV421. Comparison of different marker expression between Lin<sup>-</sup>CD127<sup>-</sup> lymphocytes in gray (non ILC population) and Lin<sup>-</sup>CD127<sup>+</sup> lymphocytes in red (Total ILCs) for DC (CD1c and CD123), basophils, mast cells and MDSC (Fc RI, CD203c and CD33) exclusion. Antibodies used: CD123-PE-Cy7, CD1c-APC-Cy7 (Biolegend, L161), FcεRI-FITC, CD203c-FITC, CD33-FITC, CD34-FITC, cKit-APC, CD127-BV421, CRTH2-

PerCP-Cy5.5. **B)** Representative histograms comparing T-bet, GATA-3 and ROR $\gamma$ t expression between Lin<sup>-</sup>CD127<sup>-</sup> lymphocytes in gray (non ILC population) and Lin<sup>-</sup>CD127<sup>+</sup> lymphocytes in red (Total ILCs). The stainings were repeated in 3 different healthy donors with comparable results. Antibodies used are: Lin-FITC, CD127-PerCP-Cy5.5, ROR $\gamma$ t-PE (Q21-559), GATA3-PE-Cy7 (L50-822), T-bet-PE-CF594 (04-46) from BD.

**Figure 4. CD161 and NKp44.** Representative histograms of CD161 expression in total ILCs (upper part), and of NKp46 and NKp44 expression in ILC3 (lower part) gated using either the Lineage ILC Mix or the Mix 4 (purple and grey histogram respectively). The stainings were repeated in 3 different healthy donors with comparable results. MFI values are shown. Antibodies used: Lin-FITC, CD127-BV421, cKit-PE, CRTH2-PE-CF594, NKp46-PerCP-Cy5.5 (9E2), NKp44-APC (p44-8) and CD161-AmCyan (HP-3G10) from Biolegend.

**Figure 5. Specific transcription factors and cytokine production in the different ILC subsets.** **A)** Total ILCs and ILC subsets were stained as in Figure 1 using the Lineage ILC mix, then the cells were fixed and permeabilized (eBioscience™ Foxp3 / Transcription Factor Staining Buffer Set) and stained for T-bet, GATA3 and ROR $\gamma$ t expression. **B)** Total PBMCs ( $3 \times 10^6$  cells/condition) were cultured with different cytokine cocktails overnight in the presence of brefeldin A (2  $\mu$ g/ml). As negative control, PBMCs were cultured in absence of stimulating cytokines for the same period of time. IL-12, IL-15, IL-18 were used to stimulate ILC1s, IL-25, IL-33, TSLP, PGD2 to stimulate ILC2s and IL-23, IL-1 $\beta$  for ILC3s. All cytokines were use at 50 ng/ml. PGD2 was used at 100 nM. Intracellular expression of IFN- $\gamma$ , IL-13, and LT- $\alpha$  was evaluated in the 3 subsets. Antibodies used: Lin-FITC, CD127-PerCP-Cy5.5 or PE-CF594, cKit-

APC/Fire 750 (Biolegend, 104D2), CRTH2-BV421 or PerCP-Cy5.5, ROR $\gamma$ t-PE, GATA3-PE-Cy7, T-bet-PE-CF594, IFN- $\gamma$ -PE-Cy7 (4S.B3) and IL-13-APC (JES10-5A2), from BD, LT- $\alpha$ -PE (Biolegend, 359-81-11).

**Figure 6. ILC gating strategy in HD and patients.** Comparison of blood-derived total ILCs and ILC subset distribution using the Lineage ILC mix in **A)** HD vs allergic patients and **B)** HD vs acute myeloid leukemia (AML) patients (n=3 in all the conditions). Antibodies used: Lin-FITC, CD127-BV421, cKit-APC, CRTH2-PE-CF594.

**Table 1. Comparison of marker expression in human ILC subsets.**

## References

1. Kiessling R, Klein E, Pross H, Wigzell H. "Natural" killer cells in the mouse. II. Cytotoxic cells with specificity for mouse Moloney leukemia cells. Characteristics of the killer cell. *Eur J Immunol* 1975;5:117-21.
2. Mebius RE, Rennert P, Weissman IL. Developing lymph nodes collect CD4+CD3- LTbeta+ cells that can differentiate to APC, NK cells, and follicular cells but not T or B cells. *Immunity* 1997;7:493-504.
3. Spits H, Di Santo JP. The expanding family of innate lymphoid cells: regulators and effectors of immunity and tissue remodeling. *Nat Immunol* 2011;12:21-7.
4. Yokota Y, Mansouri A, Mori S, Sugawara S, Adachi S, Nishikawa S, Gruss P. Development of peripheral lymphoid organs and natural killer cells depends on the helix-loop-helix inhibitor Id2. *Nature* 1999;397:702-6.
5. Cherrier M, Sawa S, Eberl G. Notch, Id2, and RORgammat sequentially orchestrate the fetal development of lymphoid tissue inducer cells. *J Exp Med* 2012;209:729-40.
6. Spits H, Cupedo T. Innate lymphoid cells: emerging insights in development, lineage relationships, and function. *Annu Rev Immunol* 2012;30:647-75.
7. Spits H, Artis D, Colonna M, Diefenbach A, Di Santo JP, Eberl G, Koyasu S, Locksley RM, McKenzie AN, Mebius RE and others. Innate lymphoid cells--a proposal for uniform nomenclature. *Nat Rev Immunol* 2013;13:145-9.
8. Shih HY, Sciume G, Poholek AC, Vahedi G, Hirahara K, Villarino AV, Bonelli M, Bosselut R, Kanno Y, Muljo SA and others. Transcriptional and epigenetic networks of helper T and innate lymphoid cells. *Immunol Rev* 2014;261:23-49.
9. Sun JC, Lanier LL. NK cell development, homeostasis and function: parallels with CD8(+) T cells. *Nat Rev Immunol* 2011;11:645-57.
10. Mebius RE. Organogenesis of lymphoid tissues. *Nat Rev Immunol* 2003;3:292-303.
11. Tait Wojno ED, Artis D. Innate lymphoid cells: balancing immunity, inflammation, and tissue repair in the intestine. *Cell Host Microbe* 2012;12:445-57.
12. Diefenbach A, Colonna M, Koyasu S. Development, differentiation, and diversity of innate lymphoid cells. *Immunity* 2014;41:354-365.
13. Yang Q, Li F, Harly C, Xing S, Ye L, Xia X, Wang H, Wang X, Yu S, Zhou X and others. TCF-1 upregulation identifies early innate lymphoid progenitors in the bone marrow. *Nat Immunol* 2015;16:1044-50.
14. Mora-Velandia LM, Castro-Escamilla O, Mendez AG, Aguilar-Flores C, Velazquez-Avila M, Tussie-Luna MI, Tellez-Sosa J, Maldonado-Garcia C, Jurado-Santacruz F, Ferat-Orsorio E and others. A Human Lin- CD123+ CD127low Population Endowed with ILC Features and Migratory Capabilities Contributes to Immunopathological Hallmarks of Psoriasis. *Front Immunol* 2017;8:176.
15. Dadi S, Chhangawala S, Whitlock BM, Franklin RA, Luo CT, Oh SA, Toure A, Pritykin Y, Huse M, Leslie CS and others. Cancer Immunosurveillance by Tissue-Resident Innate Lymphoid Cells and Innate-like T Cells. *Cell* 2016;164:365-77.
16. Fuchs A, Vermi W, Lee JS, Lonardi S, Gilfillan S, Newberry RD, Cella M, Colonna M. Intraepithelial type 1 innate lymphoid cells are a unique subset of IL-12- and IL-15-responsive IFN-gamma-producing cells. *Immunity* 2013;38:769-81.
17. Valitutti S, Muller S, Cella M, Padovan E, Lanzavecchia A. Serial triggering of many T-cell receptors by a few peptide-MHC complexes. *Nature* 1995;375:148-51.
18. Rasmussen SM, Bilgrau AE, Schmitz A, Falgreen S, Bergkvist KS, Tramm AM, Baech J, Jacobsen CL, Gaihede M, Kjeldsen MK and others. Stable phenotype of B-cell subsets following cryopreservation and thawing of normal human lymphocytes stored in a tissue biobank. *Cytometry B Clin Cytom* 2015;88:40-9.

19. Sonnenberg GF, Mjosberg J, Spits H, Artis D. SnapShot: innate lymphoid cells. *Immunity* 2013;39:622-622 e1.
20. Autissier P, Soulas C, Burdo TH, Williams KC. Evaluation of a 12-color flow cytometry panel to study lymphocyte, monocyte, and dendritic cell subsets in humans. *Cytometry A* 2010;77:410-9.
21. Tsuda J, Li W, Yamanishi H, Yamamoto H, Okuda A, Kubo S, Ma Z, Terada N, Tanaka Y, Okamura H. Involvement of CD56brightCD11c+ cells in IL-18-mediated expansion of human gammadelta T cells. *J Immunol* 2011;186:2003-12.
22. Chirumbolo S. State-of-the-art review about basophil research in immunology and allergy: is the time right to treat these cells with the respect they deserve? *Blood Transfus* 2012;10:148-64.
23. Gabrilovich DI, Ostrand-Rosenberg S, Bronte V. Coordinated regulation of myeloid cells by tumours. *Nat Rev Immunol* 2012;12:253-68.
24. Hazenberg MD, Spits H. Human innate lymphoid cells. *Blood* 2014;124:700-9.
25. Mjosberg JM, Trifari S, Crellin NK, Peters CP, van Drunen CM, Piet B, Fokkens WJ, Cupedo T, Spits H. Human IL-25- and IL-33-responsive type 2 innate lymphoid cells are defined by expression of CCR4 and CD161. *Nat Immunol* 2011;12:1055-62.
26. Lim AI, Li Y, Lopez-Lastra S, Stadhouders R, Paul F, Casrouge A, Serafini N, Puel A, Bustamante J, Surace L and others. Systemic Human ILC Precursors Provide a Substrate for Tissue ILC Differentiation. *Cell* 2017;168:1086-1100 e10.
27. Trabanelli S, Curti A, Lecciso M, Salome B, Riether C, Ochsenbein A, Romero P, Jandus C. CD127+ innate lymphoid cells are dysregulated in treatment naive acute myeloid leukemia patients at diagnosis. *Haematologica* 2015;100:e257-60.
28. Chevalier MF, Trabanelli S, Racle J, Salome B, Cesson V, Gharbi D, Bohner P, Domingos-Pereira S, Dartiguenave F, Fritschel AS and others. ILC2-modulated T cell-to-MDSC balance is associated with bladder cancer recurrence. *J Clin Invest* 2017;127:2916-2929.
29. Del Zotto G, Marcenaro E, Vacca P, Sivori S, Pende D, Della Chiesa M, Moretta F, Ingegnere T, Mingari MC, Moretta A and others. Markers and function of human NK cells in normal and pathological conditions. *Cytometry B Clin Cytom* 2017;92:100-114.
30. Vallentin B, Barlogis V, Piperoglou C, Cypowyj S, Zucchini N, Chene M, Navarro F, Farnarier C, Vivier E, Vely F. Innate Lymphoid Cells in Cancer. *Cancer Immunol Res* 2015;3:1109-14.
31. Bal SM, Bernink JH, Nagasawa M, Groot J, Shikhagaie MM, Golebski K, van Drunen CM, Lutter R, Jonkers RE, Hombrink P and others. IL-1beta, IL-4 and IL-12 control the fate of group 2 innate lymphoid cells in human airway inflammation in the lungs. *Nat Immunol* 2016;17:636-45.
32. Cording S, Medvedovic J, Aychek T, Eberl G. Innate lymphoid cells in defense, immunopathology and immunotherapy. *Nat Immunol* 2016;17:755-7.
33. Bernink JH, Peters CP, Munneke M, te Velde AA, Meijer SL, Weijer K, Hreggvidsdottir HS, Heinsbroek SE, Legrand N, Buskens CJ and others. Human type 1 innate lymphoid cells accumulate in inflamed mucosal tissues. *Nat Immunol* 2013;14:221-9.
34. Oliphant CJ, Hwang YY, Walker JA, Salimi M, Wong SH, Brewer JM, Englezakis A, Barlow JL, Hams E, Scanlon ST and others. MHCII-mediated dialog between group 2 innate lymphoid cells and CD4(+) T cells potentiates type 2 immunity and promotes parasitic helminth expulsion. *Immunity* 2014;41:283-95.
35. Kim BS, Wojno ED, Artis D. Innate lymphoid cells and allergic inflammation. *Curr Opin Immunol* 2013;25:738-44.
36. Sawa S, Lochner M, Satoh-Takayama N, Dulauroy S, Berard M, Kleinschek M, Cua D, Di Santo JP, Eberl G. RORgammat+ innate lymphoid cells regulate intestinal homeostasis by integrating negative signals from the symbiotic microbiota. *Nat Immunol* 2011;12:320-6.
37. Goncalves P, Di Santo JP. An Intestinal Inflammasome - The ILC3-Cytokine Tango. *Trends Mol Med* 2016;22:269-71.
38. Halim TY, Krauss RH, Sun AC, Takei F. Lung natural helper cells are a critical source of Th2 cell-type cytokines in protease allergen-induced airway inflammation. *Immunity* 2012;36:451-63.



39. Bartemes KR, Kephart GM, Fox SJ, Kita H. Enhanced innate type 2 immune response in peripheral blood from patients with asthma. *J Allergy Clin Immunol* 2014;134:671-678 e4.
40. Barnig C, Cernadas M, Dutile S, Liu X, Perrella MA, Kazani S, Wechsler ME, Israel E, Levy BD, Lipoxin A4 regulates natural killer cell and type 2 innate lymphoid cell activation in asthma. *Sci Transl Med* 2013;5:174ra26.
41. Wojno ED, Monticelli LA, Tran SV, Alenghat T, Osborne LC, Thome JJ, Willis C, Budelsky A, Farber DL, Artis D. The prostaglandin D(2) receptor CRTH2 regulates accumulation of group 2 innate lymphoid cells in the inflamed lung. *Mucosal Immunol* 2015;8:1313-23.
42. Pettipher R, Whittaker M. Update on the development of antagonists of chemoattractant receptor-homologous molecule expressed on Th2 cells (CRTH2). From lead optimization to clinical proof-of-concept in asthma and allergic rhinitis. *J Med Chem* 2012;55:2915-31.
43. van Beek JJP, Martens AWJ, Bakdash G, de Vries IJM. Innate Lymphoid Cells in Tumor Immunity. *Biomedicines* 2016;4.
44. Mattner J, Wirtz S. Friend or Foe? The Ambiguous Role of Innate Lymphoid Cells in Cancer Development. *Trends Immunol* 2017;38:29-38.
45. Munneke JM, Bjorklund AT, Mjosberg JM, Garming-Legert K, Bernink JH, Blom B, Huisman C, van Oers MH, Spits H, Malmberg KJ and others. Activated innate lymphoid cells are associated with a reduced susceptibility to graft-versus-host disease. *Blood* 2014;124:812-21.
46. de Weerd I, van Hoeven V, Munneke JM, Endstra S, Hofland T, Hazenberg MD, Kater AP. Innate lymphoid cells are expanded and functionally altered in chronic lymphocytic leukemia. *Haematologica* 2016;101:e461-e464.
47. Hoorweg K, Peters CP, Cornelissen F, Aparicio-Domingo P, Papazian N, Kazemier G, Mjosberg JM, Spits H, Cupedo T. Functional Differences between Human NKp44(-) and NKp44(+) RORC(+) Innate Lymphoid Cells. *Front Immunol* 2012;3:72.
48. Perry JS, Han S, Xu Q, Herman ML, Kennedy LB, Csako G, Bielekova B. Inhibition of LT $\alpha$  cell development by CD25 blockade is associated with decreased intrathecal inflammation in multiple sclerosis. *Sci Transl Med* 2012;4:145ra106.
49. Walker JA, Barlow JL, McKenzie AN. Innate lymphoid cells--how did we miss them? *Nat Rev Immunol* 2013;13:75-87.

	ILC1	ILC2	ILC3 NCR <sup>+</sup>	ILC3 NCR <sup>-</sup>	Lineage
CD3	-	-	-	-	
CD4	-	-	-	-	
CD8	-	-	-	-	
CD14	-	-	-	-	
CD15	-	-	-	-	
CD16	-	-	-	-	
CD19	-	-	-	-	
CD20	-	-	-	-	
CD33	-	-	-	-	
CD34	-	-	-	-	
CD203c	-	-	-	-	
FcεRI	-	-	-	-	
CD127	+	+	+	+	
CRTH2 (CD294)	-	+	-	-	
cKit (CD117)	-	+/-	+	+	
CD56	-	-	+/-	+/-	
NKp46	-	-	+	-	
NKp44	-	-	+/-	-	
NKp30	-	-	+/-	+/-	
CD161	+/-	+	+	+	
CD11c	-	-	+/-	+/-	
PD-1 (CD279)	-	-	-	-	
CTLA-4 (CD152)	-	-	-	-	
TIM-3	-	-	+/-	-	

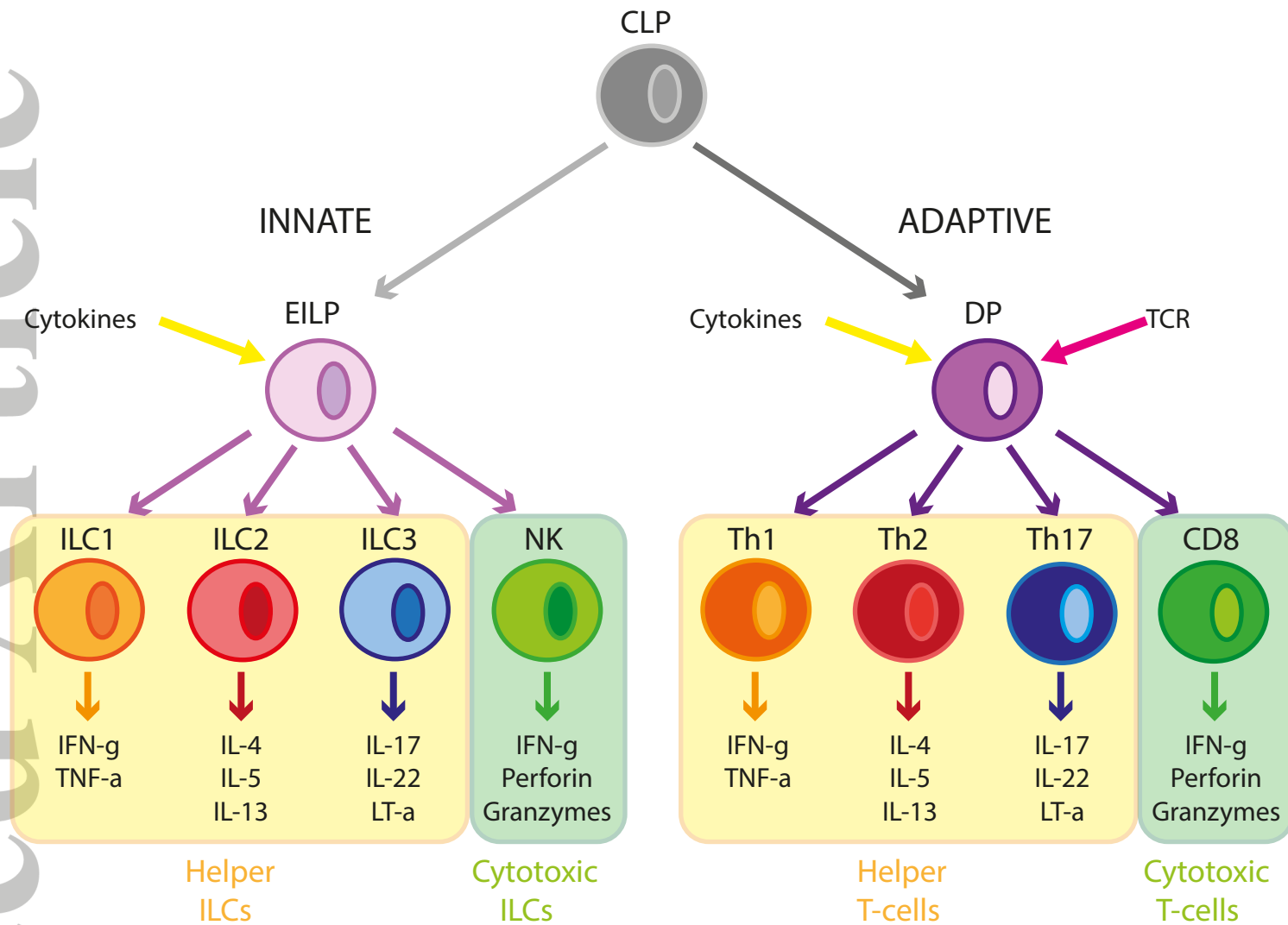


Figure 2

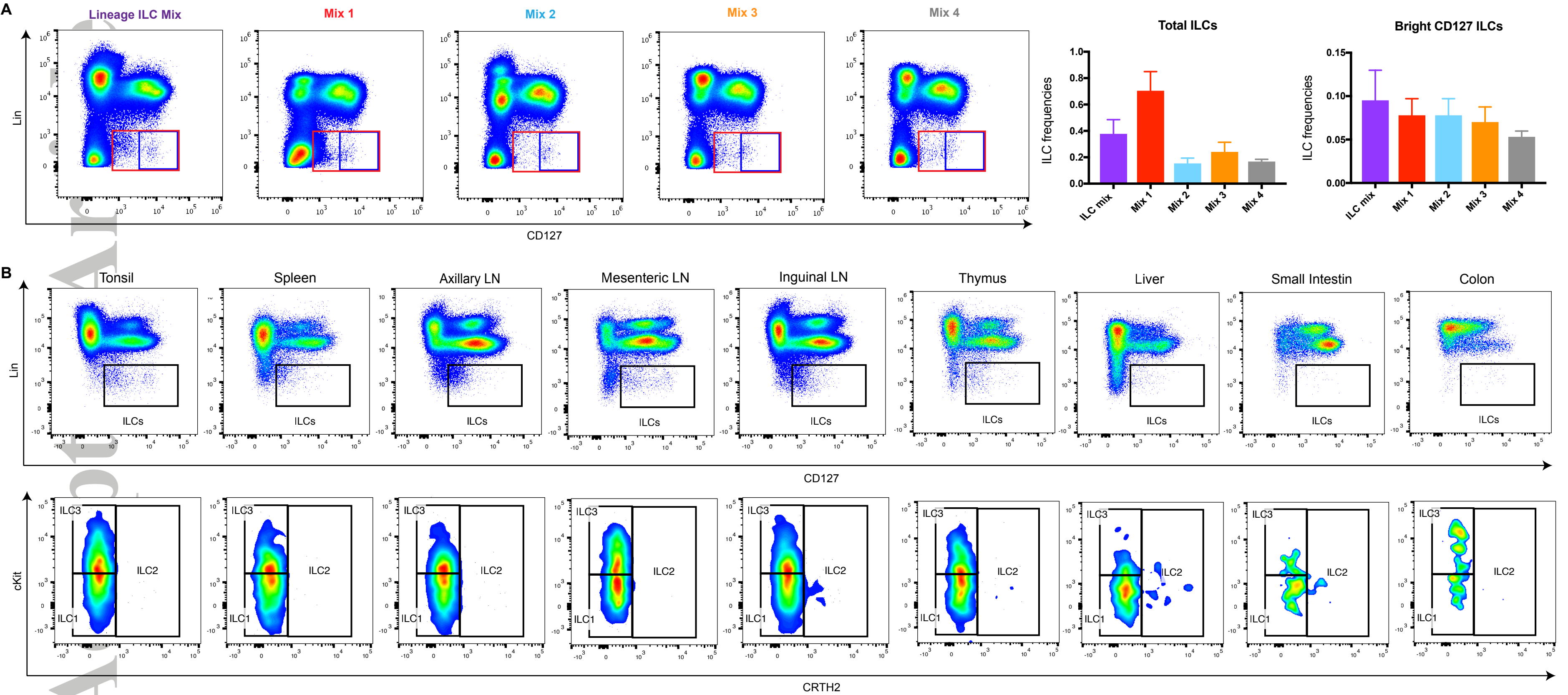


Figure 3

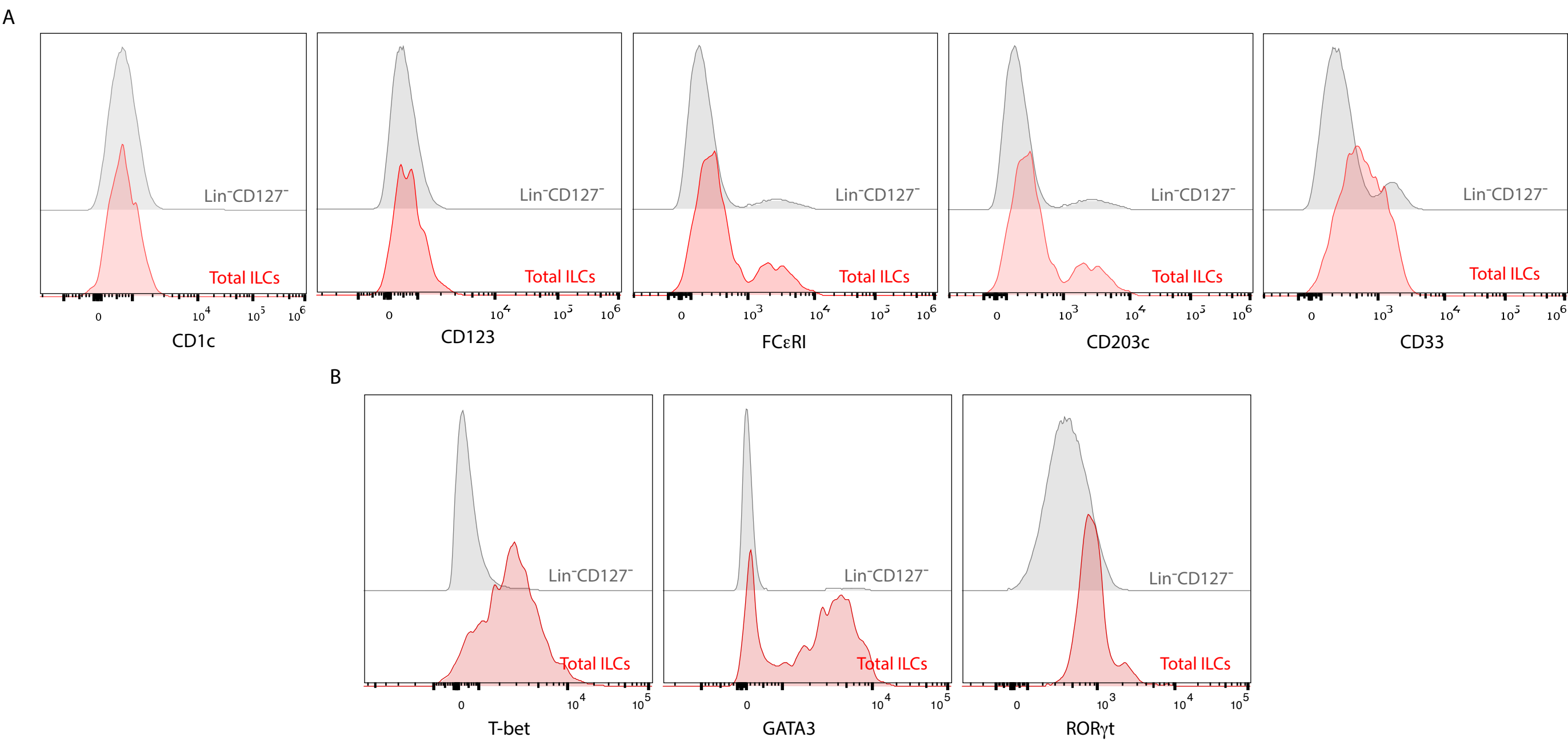
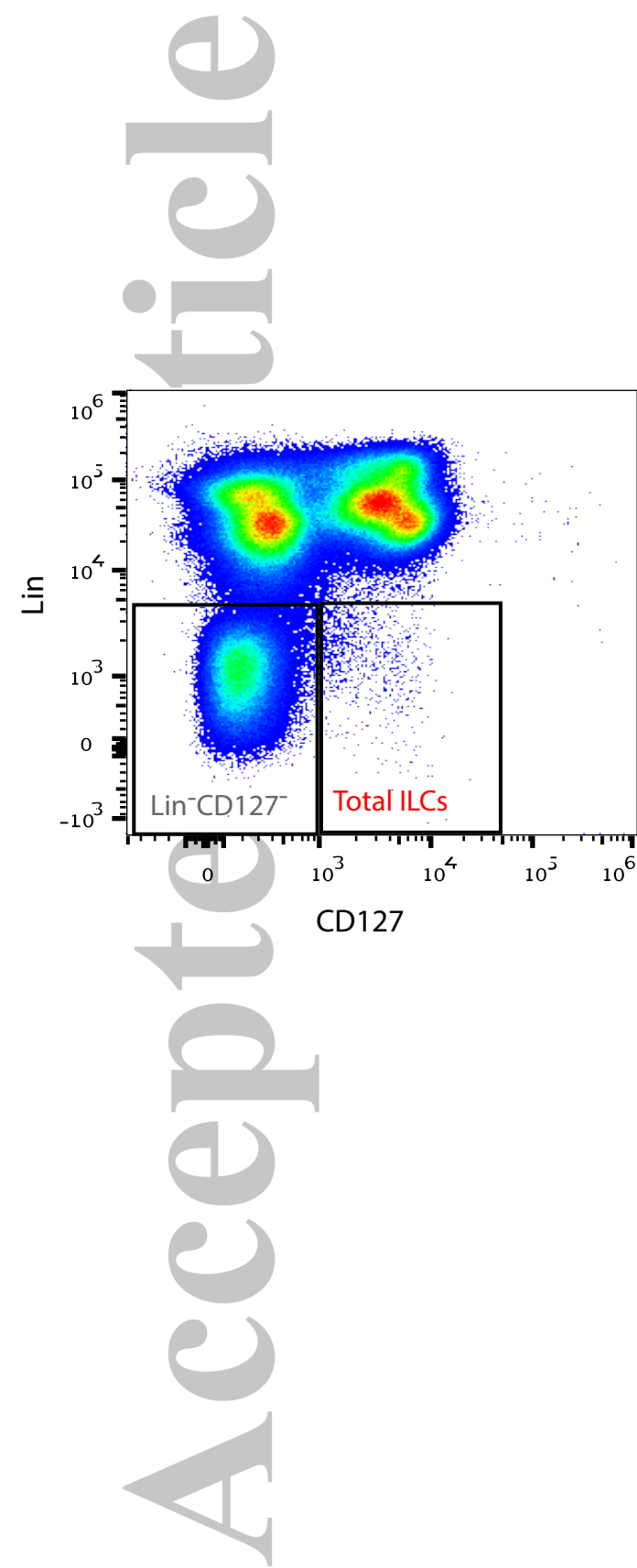


Figure 4

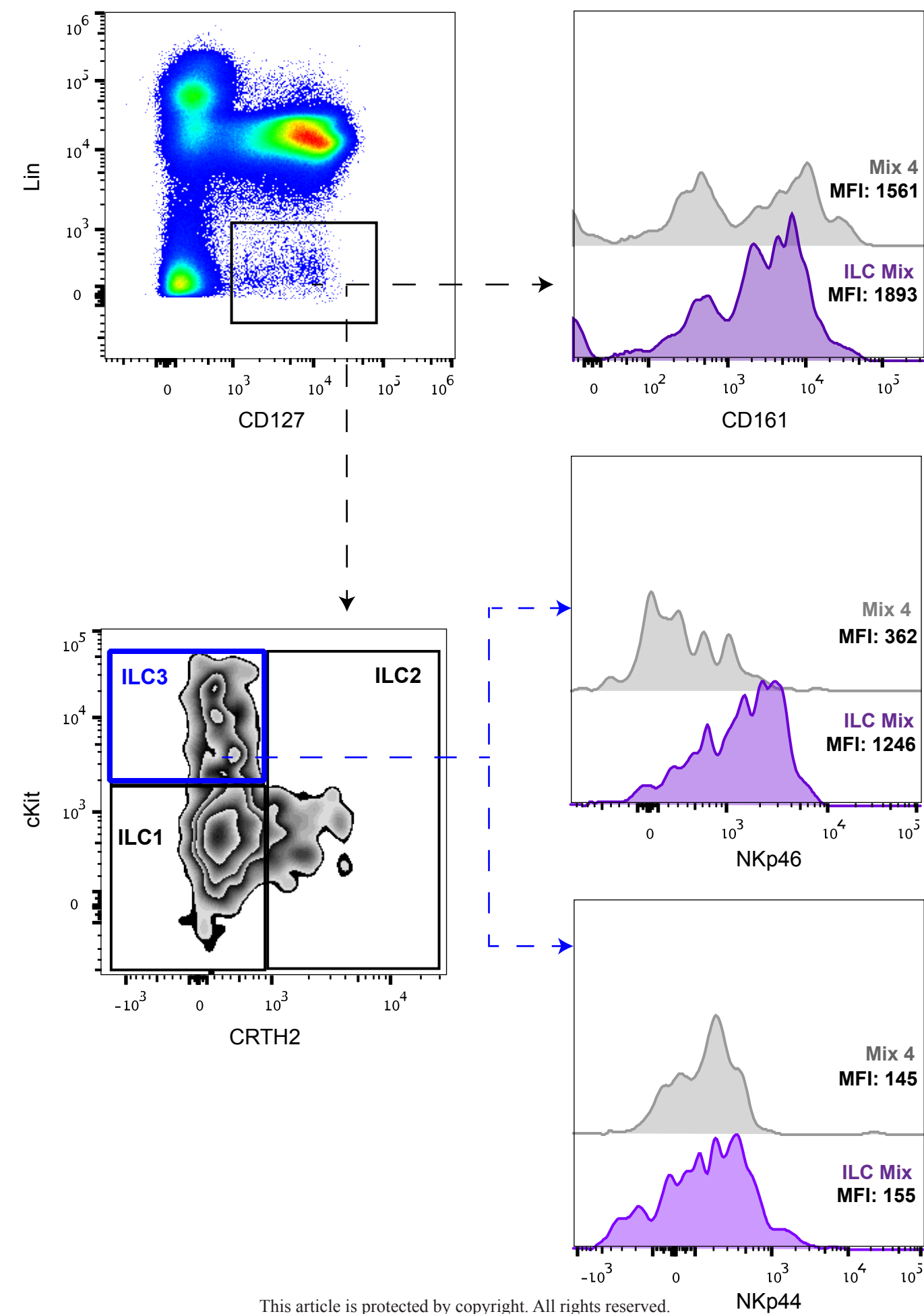


Figure 5

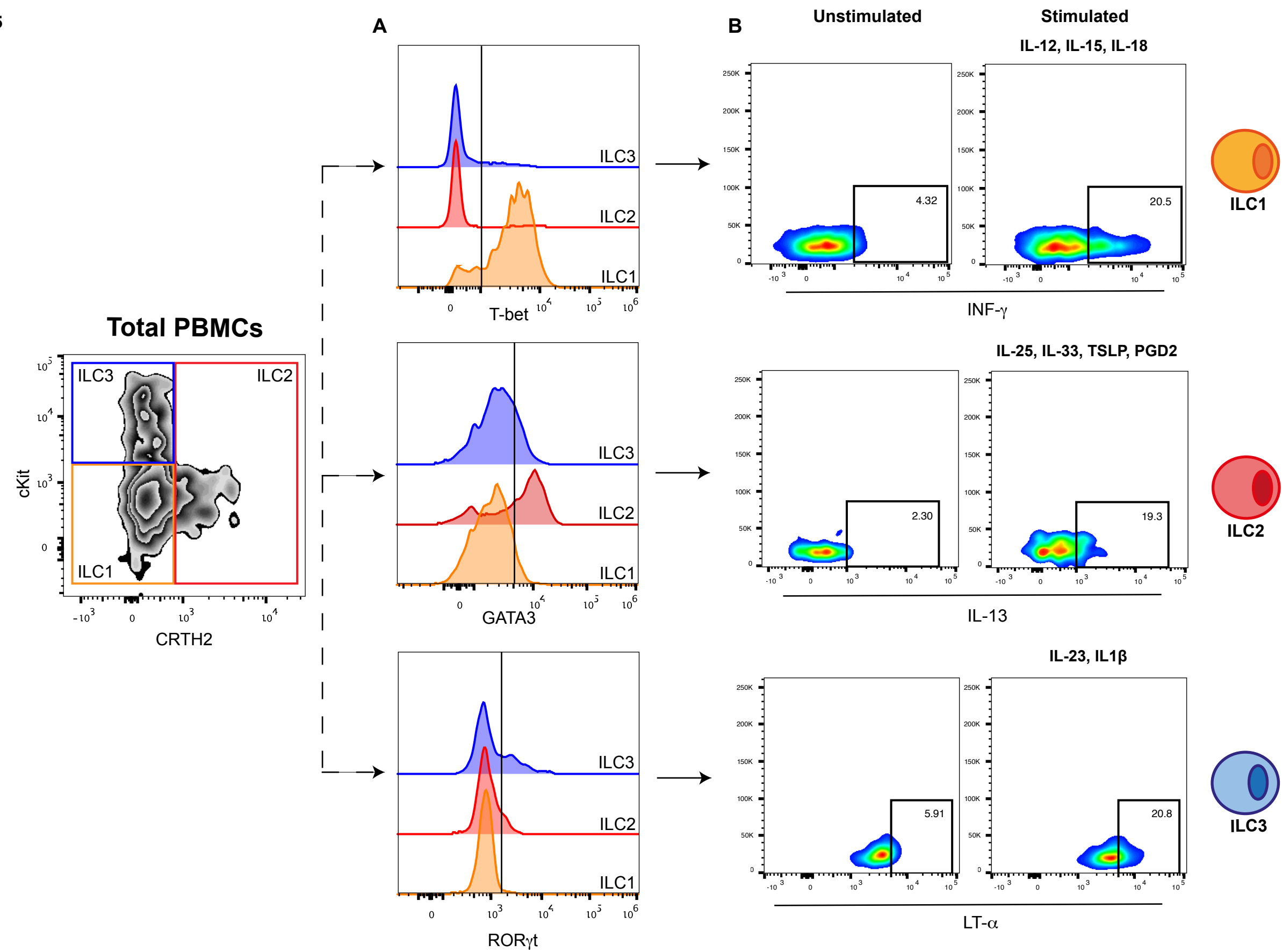




Figure 6

